THE OCCURRENCE AND PROPERTIES OF THREONINE

BY

DONALD THEODORE WARNER

A. B., Hope College, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE SCHOOL OF THE UNIVERSITY
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ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Professor Wm. C. Rose, who suggested these problems and continually contributed his advice and encouragement, and to Doctor C. S. Vestling, who generously assisted in the determination of the isoelectric point of threonine by his interest and cooperation.

THE OCCURRENCE AND PROPERTIES OF THREONINE

PART I

The Isoelectric Point of Threonine and some Related Compounds*

INTRODUCTION

The isoelectric points of amino acids can be most readily determined from the dissociation constants, K'_1 and K'_2, of the acidic and basic groups.[2] The experimental evaluation of these constants involves the measurement of the pH of solutions of the amino acids after adding varying amounts of acid (to determine K'_1 values) or of base (to determine K'_2 values). From theoretical considerations, the pH can be related to these constants by means of the following equations:

$$pK'_1 = pH - \log \left(\frac{C}{A - [H^+]} - 1 \right)$$

$$pK'_2 = pH + \log \left(\frac{C}{B - [OH^-]} - 1 \right)$$

C expresses the concentration of the amino acid under consideration, and B and A represent the total base and total acid respectively.

From the values of pK'_1 and pK'_2, the isoelectric point can be determined by the equation, $pI' = \frac{1}{2} (pK'_1 + pK'_2)$. By the use of this method we have determined the isoelectric points and dissociation constants of *l*-threonine,*dl*-allothreonine,*dl*-O-methylthreonine, *dl*-O-methylallothreonine, and *dl*-α-amino-n-butyric acid.

EXPERIMENTAL

For each of the amino acids studied, a 0.1 *M* solution of the compound in 0.1 *M* sodium chloride was prepared, and portions of this solution were treated with 0.095 *M* hydrochloric acid (to determine pK'_1 values) or with 0.0993 *M* sodium hydroxide solution (to determine pK'_2 values). Thus the ionic strength was maintained at nearly 0.1 *M* for each determination. After the

*Cf. Vestling and Warner.[1]

addition of the acid or alkali the pH of each mixture was recorded with a standardized Beckman pH meter at 25°C. From these values, pK'_1, pK'_2, and pI′ were calculated.

The results for the pI′ values were as follows: *l*-threonine, 5.59; *dl*-allothreonine, 5.56; *dl*-O-methylthreonine, 5.51; *dl*-O-methylallothreonine, 5.41; and *dl*-*α*-amino-n-butyric acid, 5.98. The pK'_1 values varied slightly for each member of the threonine group of compounds, but the pK'_2 values were nearly identical. However, the pK'_2 value for *α*-amino-n-butyric acid was very different. The direction of this variation indicates that the negative *β*-substituent in the threonine group of compounds decreases the basicity of the adjacent amino group, or makes it a stronger "acidic group".

SUMMARY

The isoelectric points and apparent dissociation constants of *l*-threonine, *dl*-allothreonine, *dl*-O-methylthreonine, *dl*-O-methylallothreonine, and *dl*-*α*-amino-n-butyric acid have been determined by electrometric titration at 25°C. and ionic strength 0.1.

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PART II

Chemical Determination of the Threonine Content of various Animal and Vegetable Proteins and a Comparison of the Values with those obtained by the Growth Method

INTRODUCTION

In order to obtain estimates of the threonine content of various proteins, Smith[1] and Bonner[2] used the growth method with young rats. This method depends upon the fact that threonine is an essential amino acid, and when it is fed at sub-optimum levels there is a definite relationship between the rate of growth and the amount of threonine present in the diet.

The Shinn-Nicolet[3] procedure for the determination of threo-

nine is a chemical method employing the reaction of this amino acid with periodic acid to produce acetaldehyde. This acetaldehyde is aerated into neutral bisulfite, and the quantitative estimation of the "bound bisulfite" is carried out with an iodine titration. Other hydroxyamino acids do not interfere.

The present problem involved an attempt to find readily available sources of threonine, and to measure the nutritional value of the various proteins with regard to their threonine content. The Shinn-Nicolet procedure gives rapid and excellent results in these studies.

EXPERIMENTAL

Threonine determinations were carried out in all cases on the dried proteins hydrolyzed with 20 per cent hydrochloric acid. After removing the excess hydrochloric acid by repeated vacuum distillation, the residue was dissolved in water and filtered. Aliquot portions were analyzed for threonine and nitrogen.

The results indicate that most of the proteins contained about 3-5 percent of threonine. In this group were found glycinin, edestin, fish protein, meat protein, egg albumin, lactalbumin, casein, serum albumin, and rabbit hair. Gliadin, peanut protein, and zein contained only 2-3 percent threonine. Three good sources of threonine were blood fibrin (5.8 percent), human hair (6.3 percent), and horse pseudoglobulin (8.0 percent).

A comparison of the threonine content obtained by the chemical method with values secured by the growth technique indicates a very satisfactory agreement in most cases.

SUMMARY

The Shinn-Nicolet procedure has been applied to the determination of the threonine content of a number of proteins. Human hair contains enough threonine to be a useful starting material in the isolation procedure. Pseudoglobulin was the richest source of threonine found in this investigation.

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PART III

Attempts to Isolate Threonine from Blood Fibrin and Hair Hydrolysates

INTRODUCTION

The isolation of pure threonine from blood fibrin was first described in the literature by McCoy, Meyer, and Rose.[1] The procedure involved a large number of butyl alcohol extractions and fractional precipitations, and the overall yields were very low.

The present investigation was undertaken to improve this isolation method. Studies have been made of the following separation procedures: the fractionation of hydrolysates with copper salts, the use of immiscible solvents, electrolytic separations, and salt formation of the amino acids with other organic or inorganic acids.

EXPERIMENTAL

The copper salt fractionation of hydrolysates was attempted because threonine forms a water-soluble copper salt, and therefore it seemed probable that other amino acids forming water-insoluble copper salts might be removed. However, when portions of a neutral hydrolysate were treated with an excess of copper sulfate or copper chloride, it was discovered that the nitrogen content of the insoluble copper salt precipitate was only slightly greater than the threonine content. Hence no useful separation was accomplished.

The extractive properties of n-butyl and n-propyl alcohol have been studied. Under similar conditions, n-propyl alcohol (8 extractions) accomplished the removal of 80-85 percent of the threonine and 50-55 percent of the nitrogen from a hydrolysate, while butyl alcohol removed 27-30 percent of the threonine and about 25 percent of the nitrogen. These results indicate the superiority of n-propyl alcohol in effectively concentrating the threonine in a particular fraction.

Electrolysis of hydrolysates in a three-compartment cell causes a separation of amino acids into basic, dicarboxylic, and neutral amino acids. The best separations obtained involved the transfer of 19-23 per cent of the nitrogen from the center compartment with a migration of about 5-6 percent of the threonine. These experiments are not easily adaptable to routine use.

Phosphotungstic acid (30 percent solution in 5 percent sulfuric acid) has been used to precipitate about 25 percent of non-threonine nitrogen from some of the "propyl alcohol-extracted material" obtained above. The filtrate from the phosphotungstic acid precipitate was then treated with 5-nitrobarbituric acid, and an additional 14 percent of the nitrogen was removed together with about 5 percent of the threonine.

SUMMARY

By subjecting a protein hydrolysate to propyl alcohol extractions and subsequently treating the extracted material with phosphotungstic acid and 5-nitrobarbituric acid, a concentrate has been obtained which contains approximately 20-25 percent of the nitrogen in the form of threonine.

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PART IV

The Nutritional Significance of certain Amino Acids in Man

INTRODUCTION

The classification of amino acids into dispensable and indispensable groups has been carried out in humans by nitrogen balance studies. With diets containing most of the nitrogen in the form of pure amino acids, the indispensability of valine and methionine have been previously demonstrated by this method.[1]

The present investigation is a determination of the human requirements for phenylalanine and threonine.

EXPERIMENTAL

Three healthy male adults were employed in this investigation. For each subject the diet furnished 7.00-7.02 gms. of nitrogen per day. Of this quantity approximately 95 percent was in the form of a mixture of nine amino acids.* The calorific requirements were supplied on the basis of 55 calories per kilogram of body weight, mostly in the form of purified carbohydrates and fats. An adequate vitamin intake was maintained by the administration of cod liver oil, crystalline thiamine, riboflavin, pyridoxine hydrochloride, nicotinamide, ascorbic acid, calcium pantothenate, α-tocopherol, and 2-methyl-1,4-naphthoquinone. Additional undetermined vitamin factors were supplied from a liver powder extract.

The complete diet served to maintain positive nitrogen balance in each subject over a five day fore-period when the nitrogen intake level was 7.00-7.02 gms. Threonine was then removed from the ration, and the remaining amino acids were increased so that the diet continued to supply a constant nitrogen intake. Immediately the two men exhibited negative nitrogen balances, and in 4-6 days the output exceeded the intake by 1.01 and 1.77 gms. respectively. The return of threonine to the diet restored the individuals to a condition of nitrogen equilibrium.

The results with phenylalanine were similar. After a preliminary nitrogen equilibrium period, the removal of phenylalanine from the ration produced negative balances of 2.74 and 2.17 gms. in the two subjects after a 4-5 day test period. The administration of the complete diet containing phenylalanine restored positive nitrogen balances in the after-period.

SUMMARY

The omission of threonine or phenylalanine from the complete diet produces definite negative nitrogen balances in adult human subjects. These amino acids are therefore indispensable constituents of the human diet.

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*One subject on the threonine experiment received ten amino acids.

VITA

The author was born April 7, 1918 in Holland, Michigan. He received his elementary and secondary education in the Holland Christian Schools. He attended Hope College from 1935 to 1939, receiving the Bachelor of Arts degree with a major in Chemistry. He entered the graduate school of the University of Illinois in the Fall of 1939, and has held a position of Special Research Assistant in Chemistry since that time.